Gluco-indole Alkaloids from *Nauclea cadamba* in Thailand and Transformation of 3α-Dihydrocadambine into the Indolopyridine Alkaloid, 16-Carbomethoxynaufoline

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Three monoterpenoid gluco-indole alkaloids, 3β-isodihydrocadambine, cadambine, and 3α-dihydrocadambine, were isolated from *Nauclea cadamba* ROXB. growing in Thailand. The stereochemistry at C19 in 3β-isodihydrocadambine was elucidated to be *R* by spectroscopic analysis. Treatment of 3α-dihydrocadambine with β-glucosidase in aqueous ammonium acetate solution gave an indolopyridine alkaloid, 16-carbomethoxynaufoline, and an unusually rearranged compound.

Key words *Nauclea cadamba*; indole alkaloid; gluco alkaloid; structure elucidation; trasformation

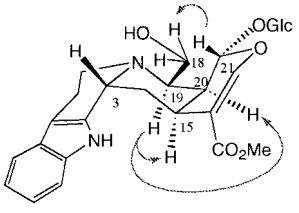
The genus *Nauclea* (Rubiaceae) is known to produce many kinds of indole alkaloids having such significant biological activites as antiproliferative, antiparasitic, and antimicrobial, for use as seed molecules for drug development. So far, investigation of the alkaloidal constituents in *Nauclea orientalis*,[1] *N. diderrichii*, *N. pobeguinii*, *N. latifolia*, *N. parva*, and *N. officinalis* have resulted in the isolation of more than fifty alkaloids, including strictosidine-related monoterpenoid indole alkaloids, indolopyridine alkaloids, *etc.* In the course of our chemical studies on the *Nauclea* alkaloids,[2—4] we investigated the chemical constituents in *Nauclea cadamba* ROXB. growing in northern Thailand. In this communication, we will describe chemically important results concerning the three isolated gluco-indole alkaloids, *i.e.*, the elucidation of the hitherto unknown stereochemistry at the C19 position of 3β-isodihydrocadambine (**1**), a characteristic phenomenon in the NMR spectra of cadambine (**2**), and chemoenzymatic first transformation of 3α-dihydrocadambine (**3**) into an indolopyridine alkaloid, 16-carbomethoxynaufoline (**5**).

The dried powdered leaves (2.7 kg) of *N. cadamba*[5] were extracted with MeOH (5 l×3 times) at room temperature. The combined filtrates were concentrated under reduced pressure to give a syrupy mass (340 g), a portion (134 g) of which was partitioned with 5% MeOH–$CHCl_3$ and water. By evaporation of the organic layer, a crude $CHCl_3$ extract (17.3 g) was obtained, which was then purified by a combination of SiO_2 flash column chromatography and medium-pressure liquid chromatography to give three gluco-indole alkaloids, 3β-dihydroisocadambine (**1**) (55 mg), cadambine (**2**) (71 mg), and 3α-dihydrocadambine (**3**) (244 mg).

The spectroscopic data (UV, IR, MS, ^{1}H- and ^{13}C-NMR, and CD) of the first alkaloid was completely identical with those of 3β-dihydroisocadambine (**1**)[6] in the literature.[7—9] However, the stereogenic center at C19 in this known alkaloid has not yet been clarified. Then, after all the protons and carbons in **1** were unambiguously assigned by ^{1}H–^{1}H correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra, we elucidated the stereochemistry at C19 by conducting differential nuclear Overhauser effect (NOE) experiments. Observation of a clear peak enhancement from H-19 (δ 3.14) to H-15 (δ 2.50) and H-20 (δ 2.07) as well as from H-21 (δ 6.05) to H-18 (δ 4.00) clearly demonstrated the *R* configuration at C19. (Fig. 1).

All the spectroscopic data (UV, IR, MS, ^{1}H- and ^{13}C-NMR, and CD) of the second alkaloid were identical with those of the well-known alkaloid, cadambine (**2**).[8,10—12] During the structural study, however, we observed an interesting phenomenon in the NMR spectra. When **2** was allowed to stand in CD_3OD, the peaks corresponding to protons (δ 2.05, 2H, m) and carbon (δ 43.0) at C14 gradually disappeared. This can be interpreted by the proton–deuterium exchange caused by ring opening at the aminoacetal position, subsequent equilibration between the imine and enamine forms,[13] and re-ring closing into the aminoacetal form (Fig. 2).

From other species of *Nauclea* plants, several indole alkaloids possessing a pyridine moiety in the molecule, such as nauclechine (**4**),[14] 16-carbomethoxynaufoline (**5**),[15] naufoline, cadamine, and naucleonidine, have been isolated. They are considered to be formed biogenetically or to be artifacts from the corresponding gluco-indole alkaloids by incorporation of ammonia into their *D*-ring. We were interested in the transformation of the major alkaloid, 3α-dihydrocadambine (**3**),[8—10,16,17] into nauclechine (**4**) or 16-carbomethoxynaufoline (**5**). Chemical or chemoenzymatic transformations of monoterpene glucosides into the corresponding monoterpenoid alkaloids having a pyridine ring have been studied. We employed a condition developed by Frederiksen and Stermitz[18] to our compound **3**. When 3α-dihydrocadambine (**3**) was treated with β-glucosidase in 10% aqueous ammonium acetate at 37 °C for 11 d, two compounds **5** and **6** could be isolated in 15% and 11% yields, respectively. The spectroscopic data of one of the two products were identical with those of 16-carbomethoxynaufoline (**5**) in the literature,[15] which was formed *via* dehydration from nauclechine (**4**) in a reaction flask. The other product **6**[19] showed UV absorption at 224, 277, and 292 (sh) nm, indicating the presence of an indole and a nicotinate residues in the molecule. The molecular formula $C_{19}H_{17}N_3O_2$ obtained by high-resolution mass spectrometry revealed that **6** lost two carbon atoms compared



3β-isodihydrocadambine (**1**)

Fig. 1. ⌒: NOE Correlations

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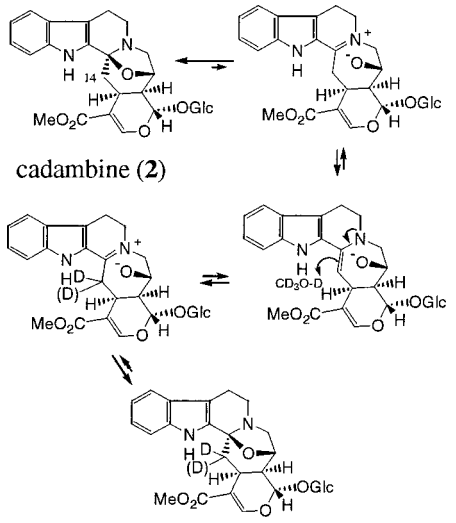

Fig. 2. A Possible Mechanism for the Proton–Deuterium Exchange at C14 in Cadambine

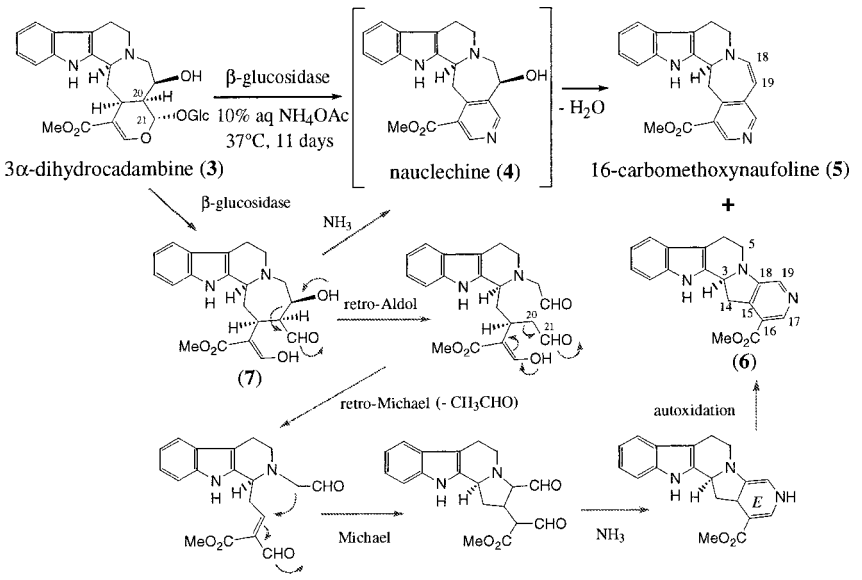

Fig. 3. Transformation of 3α-Dihydrocadambine into Indolopyridine Alkaloids

with **5**. The ^{13}C-NMR spectrum disclosed the presence of 14 sp^2 and 5 sp^3 carbon atoms, and in the ^{1}H-NMR spectrum, four aromatic protons on the indole ring, two deshielded aromatic protons on the pyridine ring, and a singlet ascribed to a carbomethoxy group were observed. Further, the presence of one isolated ethane bridge and a fragment of –CH–CH_2– was also revealed by ^{1}H–^{1}H COSY. Finally, analyzing the HMQC and HMBC[19] spectra enabled us to construct the unusual structure **6** possessing a 5,11,12,5a-tetrahydroindolo[3,2-g]pyridino[4,3-b]indolizine ring system. This new skeletal compound could be produced from aglycon **7** *via* a sequence of retro-Aldol, retro-Michael (two carbon elimination), Michael addition, incorporation of ammonia, and oxidative aromatization of the *E*-ring, as shown in Fig. 3.

Further chemical investigations on other *Nauclea* species are in progress in our laboratories.

References and Notes

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5) The leaves of *N. cadamba* were collected in northern Thailand in July and identified by J. F. Maxwell, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. A voucher specimen was deposited at the herbarium of the Faculty of Science, Chiang Mai University.
6) The detailed ^{1}H-NMR data of **1** have not been published so far; thus, we present them here. ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.52 (1H, s, H-17), 7.39 (1H, d, J=7.2 Hz, H-9), 7.34 (1H, d, J=7.2 Hz, H-12), 7.05 (1H, t, J=7.2 Hz, H-11), 6.98 (1H, t, J=7.2 Hz, H-10), 6.05 (1H, d, J=8.8 Hz, H-21), 4.83 (1H, d, J=7.9 Hz, H-1′), 4.50 (1H, br s, H-3), 4.00 (1H, dd, J=11.8, 7.4 Hz, H-18), 3.94 (1H, dd, J=11.8, 4.7 Hz, H-18), 3.91 (1H, dd, J=12.4, 2.2 Hz, H-6′), 3.74 (3H, s, OMe), 3.66 (1H, dd, J=12.4, 7.2 Hz, H-6′), 3.61 (1H, br d, J=8.5 Hz, H-5), 3.41 (1H, dd, J=9.1, 9.1 Hz, H-3′), 3.38 (1H, ddd, J=7.2, 7.2, 2.2 Hz, H-5′), 3.24 (1H, dd, J=9.1, 7.2 Hz, H-4′), 3.23 (1H, dd, J=9.1, 7.9 Hz, H-2′), 3.14 (1H, m, H-19), 3.08 (2H, m, H-6 and 5), 2.55 (1H, br d, J=10.7 Hz, H-6), 2.50 (1H, m, H-15), 2.45 (1H, m, H-14), 2.07 (1H, m, H-20), 1.88 (1H, m, H-14).
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19) Selected data of compound **6**: an amorphous powder, UV (MeOH) λ_{max} nm (log ε): 224 (4.55), 277 (4.11), 292 (sh) (3.98). CD (c 0.28 mM, MeOH, 25 °C) λ_{max} nm ($\Delta\varepsilon$): 385 (0), 354 (−1.2), 312 (0), 277 (+6.1), 250 (+1.5), 232 (+7.8), 225 (0), 214 (−11.8). EI-MS m/z: 319 $(M)^+$, 256, 129. HR-MS: Calcd for $C_{19}H_{17}N_3O_2$: 319.1320, Found 319.1325. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.51 (1H, s, H-17), 8.19 (1H, s, H-19), 7.84 (1H, br s, NH), 7.42 (1H, d, J=7.2 Hz, H-9), 7.31 (1H, d, J=7.2 Hz, H-12), 7.15 (1H, t, J=7.2 Hz, H-11), 7.07 (1H, t, J=7.2 Hz, H-10), 5.22 (1H, br d, J=9.3 Hz, H-3), 4.09 (1H, dd, J=14.6, 5.2 Hz, H-5), 3.87 (3H, s, OMe), 3.74 (1H, br d, J=17.3 Hz, H-14), 3.64 (1H, dd, J=17.3, 9.3 Hz, H-14), 3.48 (1H, m, H-5), 2.95 (1H, m, H-6), 2.69 (1H, m, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.1 (CO), 147.5 (18), 141.6 (16), 141.4 (17), 136.1 (13), 133.7 (2), 132.6 (19), 126.8 (8), 123.0 (15), 122.3 (11), 119.8 (10), 118.2 (9), 110.8 (12), 109.1 (7), 59.3 (3), 52.0 (OMe), 42.3 (5), 35.6 (14), 17.3 (6). Selected HMBC correlations: H3→C2, H5→C3, H5→C7, H14→C2, H14→C16, H17→C15, H17→C16, H19→C17, H19→C18.